Seroprevalence of feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) in shelter cats on the island of Newfoundland, Canada

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Abstract

Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are retroviruses found within domestic and wild cat populations. These viruses cause severe illnesses that eventually lead to death. Housing cats communally for long periods of time makes shelters at high risk for virus transmission among cats. We tested 548 cats from 5 different sites across the island of Newfoundland for FIV and FeLV. The overall seroprevalence was 2.2% and 6.2% for FIV and FeLV, respectively. Two sites had significantly higher seroprevalence of FeLV infection than the other 3 sites. Analysis of sequences from the FeLV *env* gene (envelope gene) from 6 positive cats showed that 4 fell within the FeLV subtype-A, while 2 sequences were most closely related to FeLV subtype-B and endogenous feline leukemia virus (en FeLV). Varying seroprevalence and the variation in sequences at different sites demonstrate that some shelters are at greater risk of FeLV infections and recombination can occur at sites of high seroprevalence.

Résumé

Le virus de l'immunodéficience féline (FIV) et le virus de la leucémie féline (FeLV) sont des rétrovirus retrouvés chez les populations de chats domestiques et sauvages. Ces virus causent des maladies sévères qui éventuellement mènent à la mort. L'hébergement de chats de façon communautaire pendant de longues périodes rend les refuges à risque élevé pour la transmission du virus parmi les chats. Nous avons testé 548 chats provenant de cinq sites différents à travers l'ile de Terre-Neuve pour FIV et FeLV. La séroprévalence globale était de 2,2 % et 6,2 % pour FIV et FeLV, respectivement. Deux sites avaient une séroprévalence significativement plus élevée d'infection par FeLV que les trois autres sites. L'analyse des séquences du gène env *de FeLV (gène de l'enveloppe) provenant de six chats positifs a montré que quatre appartenaient au sous-type A de FeLV, alors que deux séquences étaient plus apparentées au sous-type B de FeLV et du virus endogène de la leucémie féline (en FeLV). Une séroprévalence variable et la variation dans les séquences à différents sites démontrent que certains refuges sont à risque plus élevé d'infections par FeLV et que de la recombinaison peut survenir aux sites avec une séroprévalence élevée.*

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Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) cause infectious diseases in both domestic cats *(Felis catus)* and wild cat species around the world. Both viruses can be transmitted horizontally, through saliva or other body fluids (1), and vertical transmission probably also occurs (2,3). Both viruses are in the family *Retroviridae* that is responsible for equine infectious anemia, caprine arthritis encephalitis, and many other diseases (4). These viruses have positive-sense, single-stranded ribonucleic acid (RNA) genomes that integrate into the host genome after reverse transcription to double-stranded deoxyribonucleic acid (DNA) upon infection. They chronically infect immune system cells and their replication can compromise the host's immune system (2,3).

As FeLV is mainly shed oronasally in saliva and tears as well as in the feces and urine (5), activities such as biting, mutual grooming, and sharing food bowls and litter pans are modes of transmission (2,6). The virus, or antibodies, may also be transmitted from the mother to her developing offspring during pregnancy or to nursing young through infected milk (2). Transmission is most successful when there is direct contact between cats because the virus is vulnerable to disinfectants, heat, and dry conditions (2). Infection with FeLV can result in impaired bone marrow function and the development of certain forms of cancer, including lymphomas (2).

Feline immunodeficiency virus (FIV) is transferred from cat to cat primarily by saliva through deep penetrating bites (3). The penetrating bites facilitate transmission by delivering the virus to the blood, thereby bypassing the opportunity for the innate immune response to contain the virus at the site of injury. Acutely infected mothers may transmit the virus to their developing offspring during pregnancy or through infected milk when nursing young (7). However, there is evidence of high levels of FIV RNA in the supernatant of milk from an infected cat (3).

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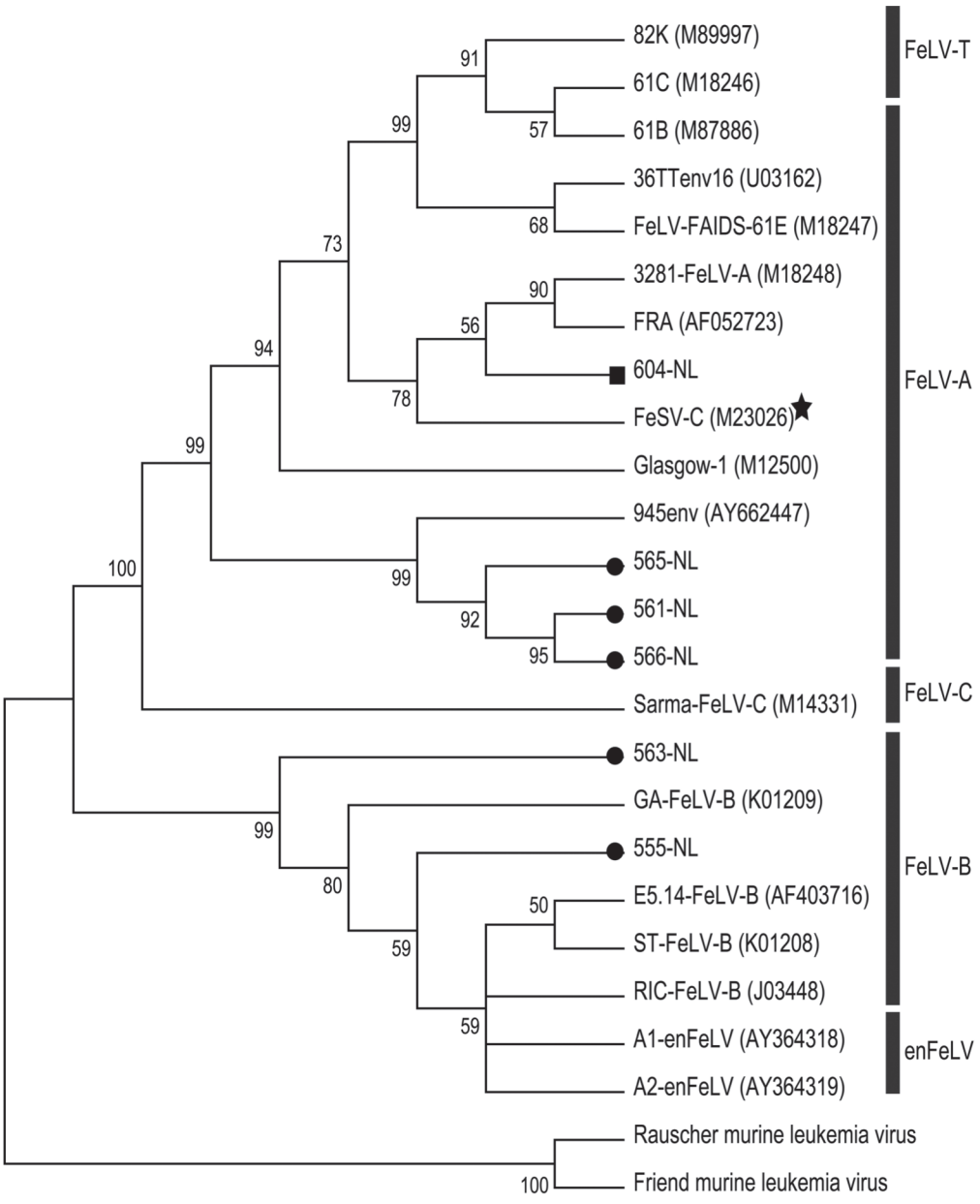

Figure 1. Phylogeny of FeLV *env* sequences. Branches labeled with filled circles represent sequences from the Newfoundland site with high seroprevalence of FeLV and the branch labeled with a filled square represents the sequence from the low seroprevalence site. Sub-types, as defined in Lutz et al (1), are indicated on the right, with the star indicating Feline Sarcoma Virus. The tree was constructed using the maximum likelihood method. Bootstrap support values based on 10 000 replicates are given at the nodes, with branches having < 50% support collapsed. Murine leukemia virus sequences (accession numbers RMU94692 and Z11128) were used as the outgroup.

The purposes of this study were: 1) to determine the seroprevalence of FeLV and FIV infections in shelter cat populations in Newfoundland, and 2) to analyze the FeLV sequences found in cats from Newfoundland. This is the first survey to focus on FIV and FeLV in animal shelters on the island of Newfoundland.

A total of 548 cats was tested for FIV and FeLV infection. Of these, 366 samples were obtained from the St. John's Humane Services, 112 from the St. John's Society for the Prevention of Cruelty to Animals (SPCA), 19 from the Clarenville SPCA, and 51 from 2 shelters in western Newfoundland. Cats of various ages, gender, ownership history, health, and breed were tested. This work was carried out under Protocol 09/10/HW from the Memorial University Institutional Animal Care Committee to HW and Biosafety Permit # S-103-1-08 to ASL.

A veterinary professional obtained blood samples from each cat either through intravenous or intracardiac extraction. Blood taken through intracardiac extraction was from heavily sedated cats that were euthanized immediately after blood collection. The blood was put into tubes either with EDTA anticoagulant or without anticoagulant from which the serum was removed and frozen. Each

Table I. Occurrence of FIV- and FeLV-seropositive cats in 5 shelters in Newfoundland. *P*-values from general linear model (GLM) analysis comparing infection status (FIV or FeLV) and shelter, age, and sex are shown

	Number of cats tested	FIV		FeLV	
		+ (%)	*P*-value	+ (%)	*P*-value
Shelter			0.46		> 0.01
Site 1	366	10 (2.7)		8 (2.2)	
Site 2	112	2 (1.8)		3 (2.7)	
Site 3	19	0		0	
Site 4	35	0		14 (40)	
Site 5	16	0		9 (56.3)	
Age			0.02		0.01
Young kitten	135	1 (0.7)		0	
Old kitten	39	0		0	
Young adult	39	0		1 (2.6)	
Adult	263	9 (3.4)		22 (8.4)	
Old adult	14	1 (7.1)		1 (7.1)	
Geriatric	26	1 (3.9)		1 (3.8)	
Unknown	32	0		9 (28.1)	
Gender			> 0.01		0.23
Females	258	1 (0.4)		10 (3.9)	
Males	261	11 (4.2)		15 (5.8)	
Unknown	29	0		9 (31.0)	
Total	548	2.2		6.2	

sample was tested for FIV and FeLV using the IDEXX SNAP FIV/FeLV Combo Test Diagnostic Kit (IDEXX Laboratories, Markham, Ontario). This kit uses enzyme-linked immunosorbent assay (ELISA) technology to detect the presence of the p27 antigen from FeLV and antibodies to the p24 antigen of FIV (8).

Statistical analysis was done in R (9). A generalized linear model was fitted with a binomial distribution and logit-link function. Feline leukemia virus (FeLV) and FIV were analyzed separately with infection status as the dependent variable and shelter, gender, and age as the independent variables. Cats were organized into 6 age groups: young kitten — birth to 11 wk; older kitten — 3 to 5 mo; young adult — 6 to 11 mo; adult — 1 to 6 y; older adult — 7 to 10 y; and geriatric — 11 y and older.

Five FeLV p27 antigen-positive samples from 1 shelter location in western Newfoundland and 1 from St. John's Humane Services were used for virus sequence analysis. Samples from the shelter in western Newfoundland were considered to come from a high seroprevalence FeLV site, whereas the sample from the St. John's Humane Services was considered to come from a lower seroprevalence site. DNA was extracted from blood samples with anticoagulant using the Qiagen Generation Capture Column Kit (Qiagen, Mississauga, Ontario) as per the manufacturer's recommendations. The DNA was then used for polymerase chain reaction (PCR) with primers that amplify from within the *env* (envelope) gene to the end of the LTR14 region (10). We modified the PCR conditions from those previously published (10) as follows: the 25-μL reactions contained 1 μL template DNA (5 to 15 ng/μL), 0.125 μL of 0.1 U/μL *Taq* DNA Polymerase (NEB, Oakville, Ontario), 2.5 μL of 10× ThermoPol reaction buffer, 0.5 μL of each 10-μM primer stock, and 0.5 μL of 10-mM dNTPs. Polymerase chain reaction (PCR) thermal cycling conditions were an initial denaturing step of 95°C for 5 min, followed by 35 cycles of 95°C denaturing for 30 s, 57°C annealing for 30 s, and 72°C extension for 2 min, and a final extension at 72°C for 5 min. Products of PCR were visualized by gel electrophoresis on a 1% agarose gel and samples with a single 2.1-kbp amplicon were purified with the QIAquick PCR Purification Kit (Qiagen) before being sequenced with the *env* primer (10). For 3 of the samples due to poor sequencing results, the PCR product was cleaned and subsequently cloned using the pGEM-T Easy Vector (Promega, Madison, Wisconsin, USA) in *Escherichia coli* NEB 5α competent cells (NEB) and sequenced from the resulting plasmids. DNA sequencing was carried out at the Centre for Applied Genomics (Toronto, Ontario). Raw sequence data were trimmed, with 726 base pair sequences from the *env* gene used for subsequent analyses. The sequences have been deposited in the GenBank database of the National Center for Biotechnology Information (NCBI) under accession numbers KC540945-KC540950.

The Newfoundland cat virus sequences were compared to known domestic cat FeLV sequences from a range of strains and subtypes (11), as indicated in Figure 1. Alignments and phylogenetic analyses were carried out with MEGA5.05 (12). The nucleotide sequences were aligned using multiple sequence alignment (MUSCLE) (13) and the evolutionary history was inferred using the maximum likelihood

method based on the Hasegawa-Kishino-Yano model (14). There were a total of 726 nucleotide positions in the final dataset and the phylogeny was bootstrapped with 10 000 replicates.

In total, 2.2% of the cats tested seropositive for FIV and 6.2% tested positive for FeLV. The seroprevalence of FIV, but not of FeLV, varied significantly between genders (Table I). Infection with FeLV, but not with FIV, varied among shelters sampled, with 2 sites having significantly higher levels of FeLV (Table I). Older cats were more likely to be positive for either FIV or FeLV.

A portion of the FeLV genome (part of the *env* gene) was amplified by PCR and sequenced from 6 of the positive samples. This included samples from 2 different locations. The resulting sequences were compared to previously reported sequences in the GenBank database by using the basic local alignment search tool (BLAST). Three of the 5 samples from the high seroprevalence site were very similar (> 99% identity) to each other. These 3 sequences were closely related (> 99% identity) to 945 *env*, a well characterized and highly virulent strain (15) within the FeLV subtype-A (FeLV-A). The sequence from the low seroprevalence site was also related to strains within FeLV-A, as well as being closely related to feline sarcoma virus, which groups within the clade containing FeLV-A. Feline sarcoma virus is a product of recombination of a FeLV-A virus with cellular oncogenes (16). The other 2 sequences from the high seroprevalence site are most closely related to endogenous proviruses and FeLV subtype-B (FeLV-B). Feline leukemia virus subtype B (FeLV-B) originated from recombination of FeLV-A with endogenous FeLV (enFeLV) (2). A phylogenetic analysis supported these BLAST results and showed that the Newfoundland sequences were not monophyletic (Figure 1).

The seroprevalence of FIV and FeLV varies among shelter populations. Past studies have found averages as low as 1.7% (17) and as high as 6.4% (18) for FIV in shelters throughout Canada. Within the Province of Newfoundland and Labrador in both shelter and privately owned cats, the seroprevalence of FeLV and FIV was previously found to be 4.33% and 5.00%, respectively (18). Of the 5 shelters we sampled, only 2 had cats that tested positive for FIV. One shelter had a seroprevalence of 1.8% and the other 2.7%, which is well within previous findings in Canada. Similar to prevalence of FIV, FeLV has ranged from 1.5% (8) to 2.7%, depending on the study (18). Four of the 5 sites that we sampled had 1 or more FeLV-positive cats and the seroprevalence ranged from 2.2% to 56.3%. The remarkably high percentage of positives was found at a site with a high-density cat population and poor sanitation, which further supports the need for proper hygienic practices to limit the spread of FeLV within shelters through testing, prevention, and responding appropriately to positive cats.

Preventative methods are usually high in most local shelters due to the routine testing of cats and strict protocols for cleaning potentially contaminated surfaces and equipment (8). However, FeLV can easily spread through an enclosed population because it is readily transmitted among cats and it takes up to 30 d for ELISA-based tests to accurately diagnose infection (8), which delays the ability to detect and react to infection. Another factor that contributes to spread of the virus is the level of isolation of the cats from one another. Distressed, aggressive, and sick cats are usually kept in cages that separate them from the others, reducing the chances of disease transmission through bites and fighting. This highlights the importance of regularly testing cats in shelters with high densities of cats, maintaining proper sanitation, and following guidelines such as those set out by the American Association of Feline Practitioners (8,19). Two of the sites in this study have a high turnover rate, i.e., cats usually do not stay at either shelter for more than a few months. This limited amount of time of exposure to any asymptomatic cats may result in the lower prevalence.

In conclusion, male cats seem to be more at risk for both viruses, especially FIV. Also, those males that tested positive were mainly intact. There is a link between shelter management styles characterized by maintaining a low density cat population, isolating sick animals, paying stringent attention to proper hygiene, and having a higher turnover rate and a relatively lower prevalence of FIV and FeLV in the shelter animals. Also, for those shelters that are interested in reducing the prevalence of FIV and FeLV in the cat population and adopting out healthy cats to prospective owners, a second FIV/FeLV test would be beneficial when cats are held for long periods of time or have higher risk of contact with the disease, given the timeline of progression of these viral diseases.

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References

1. Lutz H, Addie D, Belak S, et al. Feline leukaemia. ABCD guidelines on prevention and management. J Feline Med Surg 2009;11:565–574.
2. Hartmann K. Feline leukemia virus infection. In: Greene CE, ed. Infectious Diseases of the Dog and Cat. 3rd ed. St. Louis, Missouri: Saunders, 2006:105–131.
3. Sellon R, Hartmann K. Feline immunodeficiency virus infection. In: Greene CE, ed. Infectious Diseases of the Dog and Cat. 3rd ed. St. Louis, Missouri: Saunders, 2006:131–142.
4. Stoye JP, Blomberg J, Coffin JM, et al. Retroviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, eds. Virus Taxonomy: Classification and Nomenclature of Viruses. St. Louis, Missouri: Elsevier, 2011:477–495.
5. Jarrett W, Jarrett O, Mackey L, Laird H, Hardy W, Essex M. Horizontal transmission of leukemia virus and leukemia in the cat. J Natl Cancer Inst 1973;51:833–841.

6. Gomes-Keller MA, Gönczi E, Tandon R, et al. Detection of feline leukemia virus RNA in saliva from naturally infected cats and correlation of PCR results with those of current diagnostic methods. J Clin Microbiol 2006;44:916–922.
7. O'Neil LL, Burkhard MJ, Hoover EA. Frequent perinatal transmission of feline immunodeficiency virus by chronically infected cats. J Virol 1996;70:2894–2901.
8. Levy J, Crawford C, Hartmann K, et al. 2008 American Association of Feline Practitioners' feline retrovirus management guidelines. J Feline Med Surg 2008;10:300–316.
9. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2012.
10. Athas GB, Choi B, Prabhu S, Lobelle-Rich PA, Levy LS. Genetic determinants of feline leukemia virus-induced multicentric lymphomas. Virology 1995;214:431–438.
11. Coelho FM, Bomfim MR, de Andrade Caxito F, et al. Naturally occurring feline leukemia virus subgroup A and B infections in urban domestic cats. J Gen Virol 2008;89:2799–2805.
12. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011;28:2731–2739.
13. Edgar RC. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004;32: 1792–1797.
14. Hasegawa M, Kishino H, Yano T. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 1985;22:160–174.
15. Levesque KS, Bonham L, Levy LS. flvi-1, a common integration domain of feline leukemia virus in naturally occurring lymphomas of a particular type. J Virol 1990;64:3455–3462.
16. Donner L, Fedele LA, Garon CF, Anderson SJ, Sherr CJ. McDonough feline sarcoma virus: Characterization of the molecularly cloned provirus and its feline oncogene (v-fms). J Virol 1982;41:489–500.
17. Levy JK, Scott HM, Lachtara JL, Crawford PC. Seroprevalence of feline leukemia virus and feline immunodeficiency virus infection among cats in North America and risk factors for seropositivity. J Am Vet Med Assoc 2006;228:371–376.
18. Little S, Sears W, Lachtara J, Bienzle D. Seroprevalence of feline leukemia virus and feline immunodeficiency virus infection among cats in Canada. Can Vet J 2009;50:644–648.
19. Little S, Bienzle D, Carioto L, Chisholm H, O'Brien E, Scherk M. Feline leukemia virus and feline immunodeficiency virus in Canada: Recommendations for testing and management. Can Vet J 2011;52:849–855.